simulations in tandem with DFT calculations, the quinol binding motifs to the Qo-site of bc1 complex is investigated for a range of Qo-site protonation states. The computations revealed a novel configuration of the key side groups at the Qo-site site, such as H156, Y147 and E295, that stabilize the reaction complex and provide an optimal configuration prior to the charge transfer reactions between quinol and iron-sulfur cluster of the Rieske protein. Re-arrangements in the E295 and Y147 side chains were observed in all our simulations, showing intermediate bridging hydrogen bonding between quinol and E295, not observed before. Simulations were extended to study cytochrome c2 docking to cytochrome c1, required for the removal of electrons from the bc1 complex. We found, the docking interface is characterized by a semicircular arrangement of electrostatic residues that draws the cytochrome c2 and bc1 complex into an encounter complex, a hydrophobic minimal core to facilitate electron transfer, and mobility mismatch between the bound surfaces to induce post-electron transfer undocking.

3040-Pos Board B470

Glutathione S-Transferase Kappa 1 Knockdown Exacerbates Complex-III-Mediated ROS Production in H9c2 Cardiac Cells

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Background: Mitochondria produce reactive oxygen species (ROS) that are scavenged by local antioxidant enzymes. Glutathione (GSH) is key intermediate in many of these reactions and its availability determines the antioxidant capacity of mitochondria. Glutathione S-transferases (GSTs) are known to consume GSH during xenobiotic detoxification but their involvement in ROS scavenging is less clear. Gstκ1 was originally identified as a novel mitochondrial GST but its role there remains unknown.

Objective: To examine whether loss of Gstκ1 affects the [GSSG/GSH] ratios in cytosol and mitochondria of the cardiac-derived cell line H9c2.

Methods: Knockdown of Gstκ1 (Gstκ1-KD) in H9c2 cells was achieved by transfection with siRNA. Changes in [GSSG/GSH] were monitored using genetically-encoded ratiometric sensors that localize to cytosol or mitochondria. Global oxidative stress was induced with hydrogen peroxide (HP). Mitochondrially-targeted oxidative stress was induced by the superoxide generator di-methoxy-naphtho-quinone (DMNQ) and by antimycin-A (AA) which interacts specifically with mitochondrial complex III.

Results: Treatment with HP elevatedcytosolic [GSSG/GSH] ratios equally in both control and Gstκ1-KD cells. Mitochondrial oxidative stress elicited by HP or DMNQ likewise increased mitochondrial [GSSG/GSH] and was unaffected by Gstκ1-KD. Inhibition of Complex III by AA decreased mitochondrial [GSSG/GSH] persistently in control cells. However, in Gstκ1-KD cells exposed to AA, the decrease was transient and was followed by a sustained increase in [GSSG/GSH].

Conclusions: Here we examine for the first time the role of Gstκ1 in ROS scavenging in cardiac cells. While our data suggest that Gstκ1 does not participate in the general response against exogenous ROS, they indicate that low levels of this enzyme associate with increased superoxide production from mitochondrial complex III. Our findings may provide insights for experimental models of cardiac disease where Gstκ1 expression is downregulated.

3041-Pos Board B471

Evaluation of Heme Peripheral Groups Interactions in Low-Dielectric Constant Media

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In this study, we measured the contributions of the ionization of the heme propionates to the reduction potentials of heme b and heme a (bis)N-methylimidazole complexes in various low-dielectric constant conditions. Additionally, we measured the effects of H-bond to the heme a formyl group on the reduction potential of the heme. The performed electrochemical measurements show that ionization of the heme propionates lead to the largest redox change in dichloromethane with no electrolyte. The measured reduction potential changes for heme b and heme a were - 55 and −47 mV (±10 mV) per ionized propionate, respectively. For heme a, the study demonstrates how the dielectric constant of the medium is important in the magnification of the αpKa upon redox-linked ionization of the heme propionates and their roles in the proton pump of cytochrome c oxidase. Additionally, we carried out a detailed study on the H-bonding properties of heme a model compounds (copper mono- and di-acetyl porphyrins) and the effects of the dielectric constant of the solvent on the measured dissociation constant (Kd) between various H-bond donors and the copper porphyrins. Our measurements show that H-bonds between the copper porphyrins and TFA or phenol are significant in benzene and dichloromethane, but extremely weak in the presence of acetonitrile. This study shows how the dielectric constant of the medium also plays a role in modulating the properties of heme a through H-bonding of its formyl group.

3042-Pos Board B472

Theoretical Investigation of the Primary Event in Proteorhodopsin Activation

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Retinal proteins are α-helical transmembrane proteins that have the potential for applications such as memory storage (bacteriorhodopsin) and optogenetics (channelrhodopsin). Proteorhodopsin (PR) is a recently discovered microbial retinal protein which acts as a proton pump, similar to bacteriorhodopsin. Initial activation of PR occurs when the covalently bound chromophore, retinal, absorbs a photon and undergoes an all-*trans* → 13-*cis* isomerization. Despite several similarities between bacteriorhodopsin and PR, the details of the activation process remain unclear. We investigated the photoisomerization of retinal in PR using a hybrid quantum mechanical (QM) and molecular mechanics (MM) approach. Two photoexcited states were identified. Both retinal structures have a 13-*cis*, 15-*anti* conformation. However, one does not involve a hydrogen bond between the Schiff base and a water molecule, while the other structure does, dependent on the rotational direction of the isomerization. The former is more stable than the latter by about 0.9 eV and the former also has a lower energy barrier of the photoisomerization by about 0.2 eV than the latter. Upon photoisomerization, PR reaches the former excited state, subsequently proceeding through a slow rearrangement to form the hydrogen bond between the Schiff base and water and generate the latter excited state. We believe the former structure to be indicative of the K state, with the latter structure more characteristic of an L-like state. This L-like state is spectroscopically silent and most likely extremely transient, due to the low energy barrier (0.1 eV) of the proton transfer from the Schiff base to the proton acceptor, D97. A detailed understanding of the initial events in the activation process is essential to utilizing PR as a light-driven component in potential technological applications.

3043-Pos Board B473

The Electron Transfer in Ferredoxins

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The 2[4Fe-4S]-ferredoxins are ubiquitous water-soluble electron shuttles found in all bacteria. The intramolecular "self-transfer" electron transfer between the two [4Fe-4S] clusters is an ideal model system for studying electron transfer independent of association of the donor and acceptor and may be considered as the limit of a very tightly bound complex. Thus, the effects of environmental factors on electron transfer rates can be focused on. *Clostridium acidurici* ferredoxin (CaFd) is a small (5.9 kDa, 55 residues), pseudo-symmetric protein containing two $[Fe_4S_4(SR)_4]^{2-}$ redox sites which are separated by ~12 Å, the typical distance of biological electron transfer. Molecular dynamics simulations of CaFd are performed using different force fields to test their performance. The environmental reorganization energy λ is calculated from the simulations and compared to experiment.

3044-Pos Board B474

Internal Switches Modulating Electron Flow in bc1 Complex

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Ubiquinol-cytochrome c oxidoreducase (BC1) protein complex is a smart machine which employs an internal signal transduction network, regulated by the binding of natural ligands to either Qo or Qi sites, to modulate electron transfer rates between different redox pairs. Binding of natural ligands or some inhibitors leads to local conformational changes which propagate through protein and control the conformation of key residues involved in the electron tunneling pathway. Aromatic-aromatic interactions are highly utilized in this internal network since the key residues are aromatic in nature. Molecular dynamics simulations of native BC1, natural ligand and inhibitor-bound BC1 homo-dimers in membrane were performed to investigate and compare the dynamics of those key residues in their respective environments. In addition, molecular dynamics simulations reveals the transduction pathway induced by the binding of a ligand or inhibitor at their sites. Electron tunneling calculations show that there is a substantial correlated change of the electron transfer rates between different redox pairs depending on the binding of natural ligands or inhibitors.

3045-Pos Board B475

Energetics of Lateral Membrane Proton Diffusion

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The diffusion of protons along biological membranes is vitally important for cellular energetics. The weak dependence of both migration speed and span on lipid composition suggests that proton migrate along water (Springer et al., 2011). However, it is unclear how to reconcile the apparently high proton affinity to the phase boundary (Zhang et al., 2012) with the poor proton acceptability of water, i.e. with the low pK(=0) value of water. Here we monitored the

diffusion of excess protons along the phosphatidylcholine lipid bilayer/water interface at different temperatures. The kinetics of proton arrival from a distant spot of proton release to lipid-anchored fluorescent pH-sensitive dyes indicated that the *in vitro* Gibbs activation energy $\Delta G^{\ddagger}$ for proton surface-to-bulk release harbours only a minor enthalpic constituent. We observed that more than 2/3 of $\Delta G^{\ddagger}$ are entropic in origin, which explains the high proton affinity to membranes in the absence of a potent proton acceptor. This work was supported by Grant P25981 from the Austrian Science Fund (FWF) to P.P.

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3046-Pos Board B476

Assessing the Protonation State and Dynamics of His37 in the Influenza M2 Proton Channel using Raman Spectroscopy

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The influenza M2 protein is known to shuttle protons into the virion, facilitating replication and maturation. Histidine 37 is thought to provide ''shuttling'' activity to those protons, but the protonation states of the four His37 residues in the tetrameric channel has not been definitively established. We use Raman spectroscopy to collect the frequency of a C2-D probe group on His-37 in an isotopically labeled version of the M2 transmembrane peptide. The C2-D frequency reports directly on the protonation state of selected His residues in a way that other experiments cannot. The data are used to identify His37 protonation states in various pH conditions and to report directly on the activity and dynamics of His37 with protons and water in its local environment.

3047-Pos Board B477

Effects of Laser Spot Sizes in Laser Driven Proton Therapy

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We present a numerical study of the effect of the laser spot size of a circularly polarized laser beam on the energy of quasi-monoenergetic protons suitable for particle cancer therapy in laser proton acceleration using a thin carbon-hydrogen foil. The energy dosage distributions of impinging the generated proton beams into human tissues are also simulated, compared and analyzed. The used proton acceleration scheme is a combination of laser radiation pressure and shielded Coulomb repulsion due to the carbon ions. We observe that the spot size plays a crucial role in determining the net charge of the electron-shielded carbon ion foil and consequently the efficiency of proton acceleration. Using a laser pulse with fixed input energy and pulse length impinging on a carbon-hydrogen foil, a laser beam with smaller spot sizes can generate higher energy but fewer quasi-monoenergetic protons. We studied the scaling of the proton energy with respect to the laser spot size and obtained an optimal spot size for maximum proton energy flux. Using the optimal spot size, we can generate an 80 MeV quasi-monoenergetic proton beam containing more than a hundred million protons using a laser beam with power 250 TW and energy 10 J and a target made of 90% carbon and 10% hydrogen, capable of treating tumor cells with depth up to 5 cm in human bodies.

3048-Pos Board B478

Characterization of Excited State Etheno-Fad: A Probe of the Role of Adenine in DNA Photolyase

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Using femtosecond transient absorption spectroscopy , we have characterized flavin 1,N^6 - ethenoadenine dinucleotide (ε-FAD), an analog of flavin adenine dinucleotide (FAD), to understand the role of adenine in the photoinduced electron transfer (PET) reaction between reduced FAD (*$FADH^-$) and thymine dimers during its repair in DNA photolyase. The adenine in FAD may serve as a virtual or real intermediate in the PET reaction. ε-FAD was used to modify the driving force between the adenine and its partners, providing data on parameters and processes that dictate the kinetics and pathways of electron transport. The neutral oxidized and the fully reduced anionic states of ε-FAD, FAD, and flavin mononucleotide (FMN) in free solution exhibited multi-exponential decay kinetics that reflect their excited state quenching pathways and possible conformational heterogeneity. Generally, the introduction of the ε-Ade group leads to much faster excited state decay in the reduced state. To investigate this further, the evolution of the excited state was probed in the visible, near-UV, and UVB spectral regions to determine whether PET was responsible for the short-lived excited state. The significance of these results to the role of the FAD adenine in DNA photolyase are discussed.

3049-Pos Board B479

High-Resolution Electronic Structure of the Primary Electron Acceptor A_0 of Photosystem I

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The Type I photosynthetic reaction center, Photosystem I, is an exquisitely tuned protein complex comprised of multiple polypeptide subunits and protein-bound electron-transfer (ET) cofactors. Upon illumination, the special chlorophylls, P_{700}, are photoexcited which results in the rapid formation of the charge-separated state, $P_{700}{}^{+}A_0{}^{-}$. In order to prevent charge recombination, the electron is transferred to the phylloquinone cofactor, A_1, and subsequently to three four iron-four sulfur [4Fe4S] clusters, F_A, F_B, and F_X, respectively. Through the ET reactions of PSI, the reducing equivalents that are required for the carbon fixation reactions are generated and stored as NADPH or 'biohydrogen'. The PSI reaction center displays pseudo-C_2 symmetry, such that the ET cofactors, A_0 and A_1, are duplicated in what is termed the A- and B-branch. Although ET is bidirectional along the A- and B-branch, it has been demonstrated that ET in the A-branch is highly preferred. This is thought to be due to the tuning of the redox-potential of the ET cofactors by smart matrix effects from the surrounding protein environment. This research explores the electronic structure of the primary electron acceptor A_0 in the A-branch (A_{0A}) through the use of two-dimensional (2D) hyperfine sublevel correlation (HYSCORE) spectroscopy in tandem with density functional theory calculations. The application of 2D HYSCORE spectroscopy allows for the identification of the ^{14}N atoms and protons that are magnetically interacting with the paramagnetic center of $A_{0A}{}^{-}$. The ^{14}N and ^{1}H hyperfine parameters obtained here provide a direct measure of the electron spin density distribution of $A_0{}^{-}$ in the A-branch of PSI. These are then be compared with simulations of the electronic structure of the A-branch of Photosystem I in order to better understand the mechanism of electron transfer from P_{700} to the primary electron acceptor, A_{0A}.

3050-Pos Board B480

Environmental Coupling and Population Dynamics in the PE545 Light-Harvesting Complex

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Experimentally long-lived quantum coherences have been found in the Fenna-Matthews-Olson (FMO) complex of green sulfur bacteria as well as in the phycoerythrin 545 (PE545) photosynthetic antenna system of marine algae. In the latter system the long-lived coherences are clearly visible also at ambient temperatures. A combination of classical molecular dynamics simulations, quantum chemistry and quantum dynamical calculations is employed to determine the excitation transfer dynamics in PE545. To be able to described the excitation transfer and dephasing phenomena the spectral density is a property a key importance. To this end a time series of the vertical excitation energies of the individual pigments is determined. In a subsequent step. quantum dynamical simulations are performed using the earlier QM/MM calculations as input . Employing an ensemble-averaged classical path-based wave packet dynamics, the excitation transfer dynamics between the different bilins in the PE545 complex is determined and analyzed. Furthermore, the nature of the environmental fluctuations determining the transfer dynamics is discussed.

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